

The growth of protozoa in sterilized soil II. – Dynamic of the ciliate community

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Abstract

The ciliate community was investigated in laboratory microcosms using forest litter layers treated with either propylene oxide, autoclaving or γ -irradiation and then amended with untreated soil samples. The subsequent growth of ciliates was followed for 28 days in each treatment, with regard to the dominant groups, i.e. colpodids, hypotrichs and *Drepanomonas* sp.

The result showed marked changes among the treatments. In fumigated soil, the population of colpodids increased rapidly after inoculation followed by hypotrichs 14 days later. In the autoclaved treatment, the growth of colpodids was rapid but trophozoites were observed only after 14 days. *Drepanomonas* sp. appeared from day 3 and remained only as trophozoite form. The population of hypotrichs increased after 28 days. In the irradiated treatment, colpodids were the first to grow followed by *Drepanomonas* sp. from day 3 and hypotrichs from day 7. Numbers of colpodids was never significantly different between treatments. Numbers of *Drepanomonas* sp. were always significantly larger in autoclaved humus than in the other treatments and numbers of hypotrichs were larger on day 28 in irradiated humus. The different responses of the ciliate community, especially the successions of species are discussed.

Keywords: Protozoa, Ciliophora, community dynamics, soil litter, sterilization.

La croissance des protozoaires en sol stérilisé II: Dynamique du peuplement de ciliés.

Résumé

Ce travail est la seconde partie d'une étude expérimentale sur la recolonisation d'un sol forestier par les protozoaires ciliés. La première partie avait été consacrée à l'analyse faunistique du peuplement introduit après fumigation du sol à l'oxyde de propylène, autoclavage ou irradiation par les rayons gamma. La seconde partie présente maintenant la diversification du peuplement selon les traitements en suivant les effectifs des groupes dominants: colpodes, hypotriches et *Drepanomonas* sp.

Dans le sol fumigé, le peuplement a été rapidement dominé par les colpodes qui se sont développés aussitôt après l'inoculation tandis que les hypotriches ont été observés seulement après 14 jours d'incubation. Dans le sol autoclavé, les colpodes se sont multipliés rapidement mais sont restés pendant les deux premières semaines uniquement sous forme enkystée. *Drepanomonas* sp. a été observée à partir du jour 3. Cette espèce s'est maintenue par la suite uniquement sous forme trophozoïte. Les hypotriches n'ont été présents qu'après le jour 28. Dans le sol irradié, comme précédemment, les colpodes se sont développés en premier suivis de *Drepanomonas* sp. à partir du jour 3. Par contre, les hypotriches ont dékysté plus tôt que dans les deux autres traitements à partir du jour 7. Si la diversification du peuplement a été bien marquée suivant les traitements, les effectifs ont été rarement significativement différents. Seule l'abondance de *Drepanomonas* sp. a toujours été plus élevée dans le sol autoclavé tandis que les hypotriches ont été plus nombreux uniquement le jour 28 dans le sol irradié. Ces résultats montrent qu'un peuplement se développe différemment selon les conditions édaphiques et amènent à discuter sur les successions de populations.

Mots-clés : Protozoaires, Ciliés, dynamique de peuplement, humus, stérilisation.

INTRODUCTION

The abundance and diversity of soil ciliates are influenced by changes in their habitat either as a result of natural events or human activity. Much of the literature on this topic has been summarized by Foissner (1987). Petz and Foissner (1989) found that different protozoa responded to pesticides in different ways. Recently, Lehle *et al.* (1993) showed that *Sathrophilus muscorum* grew better in soil after fertilizer amendments, but usually only 10 to 20 % of the ciliate species remained dominant (i.e. *Colpoda inflata*, *C. steinii*, *Cyclidium muscicola*) after all treatments.

Soil sterilization can produce many different physical and chemical changes that vary according to the soil type and the degree of intensity of the treatment (Enoe and Popeno, 1964; Salonius *et al.*, 1967; Skipper and Westermann, 1973). These changes can produce new environmental conditions that may either stimulate or inhibit the organisms present. For example, soil samples of a forest rendzina dried at 105°C and then returned to the forest site, had increased growth of the bacterium *Bacillus* and some collembola (Kilbertus *et al.*, 1976). Furthermore, small numbers of protozoan species recolonize soils fumigated with methyl bromide (Yeates *et al.*, 1991). The ciliate *Colpoda aspera* develops better in a soil after fumigation with propylene oxide than after autoclaving or γ -irradiation (Palka and Coûteaux, 1986). Different methods of sterilization can therefore be used to stimulate different sections of the ciliate community.

This paper is the second part of an experimental study of ciliate recolonization of soil litter sterilized with either propylene oxide, autoclaving or γ -irradiation. The first part of this study (Palka, 1991) dealt with variations in the presence or absence of species in the different treatments. Three groups of ciliates "indifferent", "semi-sensitive" and "sensitive" to the changes after sterilization were proposed. In this second paper, the changes in the ciliate community of three numerically dominant groups, colpodids, hypotrichs and *Drepanomonas sp.* are presented.

MATERIALS AND METHODS

Experimental procedure

Soil litter from the H and F layers of a dysmoder (27.8 % C, 1.3 % N, pH (H₂O) 4.7) was collected from Fontainebleau forest (France) dominated by oak (*Quercus petraea* (Mattus) Liebl.) and hornbeam (*Carpinus betulus* (L.)). This sample was passed through a 2 mm sieve and stored at 5°C before use. The experimental model was a syringe of 10 cm³ (Coûteaux, 1984) containing moist sterile soil, equivalent to 1.5 g dry weight. The sterilization

methods used for propylene oxide, autoclaving and γ -irradiation were as described by Palka and Coûteaux (1986). At the start of the experiment, 1 ml of filtered soil extract without protozoa (Millipore filter, 2 μ m diam. pores) was injected and water content was adjusted to 300 % dry weight with sterile demineralized water i.e. to pF1. Seven days later, moist untreated soil (pF 1.5) equivalent of 0.16 g of dry weight was introduced. Eighteen microcosms were prepared for each treatment and three replicates were used at each sample date i.e. 1, 3, 7, 14, 21 and 28 days after inoculation of untreated soil. Incubation was performed in the dark at 20°C.

Counts

The twofold dilution method was used with 8 dilution levels and 8 replicates per level (Darbyshire, 1973). Each microcosm was suspended in 16 ml of sterile demineralized water and homogenized using a sterile glass pipette. Then 6 ml of this suspension were added to 6 ml of sterile demineralized water. The same procedure was made for the next dilution levels. For each level, 8 replicates of 0.5 ml were pipetted with a micropipette (Socorex) and put into a sterile rigid styrene plate with 24 wells.

For each plate, ciliates present into the wells were counted using an inverted microscope (Leitz) at two occasions. The first count was made immediately after dilutions in order to estimate the number of trophozoites present at the time of sampling. After that, some drops of filtered soil extract (2 μ m diam. pores) were put into the wells containing dilutions and the plates were incubated for 3 days in the dark at 25 °C to allow excystment. Then a second count was made in order to estimate the number of cysts. Abundances were obtained by the MPN method using the statistical tables of Fisher and Yates (1943).

Results were compared for each group of ciliates using the Student's t-test at the 5 % level (StatView 512TM). The comparisons were performed for each treatment between the day 1 and 3, 3 and 7 and so on. Computations were made also between treatments.

RESULTS

Propylene oxide fumigated soil (table I)

The population of colpodids increased very rapidly after inoculation, but variation between replicate microcosms was very large during the first week, especially for cysts. Total numbers (trophozoites + cysts) were significantly different between days 14 and 21 ($p=0.0027$). On day 21, the population reached a maximum of about 30 000 cells g⁻¹ dry soil but the number of trophozoites was significantly lower than the number of cysts ($p=0.0028$) in the ratio of 1 to 27.

Table I. – Numbers of ciliates (g^{-1} dry soil) after propylene oxide fumigation and reinoculation with untreated soil. No *Drepanomonas* sp. observed. (means $\pm$ SE).

	Days					
	1	3	7	14	21	28
Colpodids						
Trophozoites	670 $\pm$ 443	1,036 $\pm$ 857	236 $\pm$ 61	342 $\pm$ 68	968 $\pm$ 384	448 $\pm$ 293
Cysts	5,920 $\pm$ 3,208	13,050 $\pm$ 10,707	2,731 $\pm$ 1,431	859 $\pm$ 269	25,709 $\pm$ 3,771	12,259 $\pm$ 5,443
Hypotrichs						
Trophozoites	0	0	0	114 $\pm$ 70	279 $\pm$ 190	54 $\pm$ 30
Cysts	0	0	0	184 $\pm$ 95	765 $\pm$ 290	188 $\pm$ 64

No hypotrichs were detected in the first week and from the second week their abundance was not significantly different whatever the date of sampling. Furthermore, differences between trophozoites numbers and cysts numbers were never significant. *Drepanomonas* sp. was not observed.

appeared after 28 days and then only as encysted forms. The population of *Drepanomonas* sp. increased from day 3 and reached a maximum of about 2500 trophozoites g^{-1} dry soil on day 21. This maximum was significantly different from the population on day 14 ($p=0.0253$). No cysts were counted.

Autoclaved soil (table II)

No trophozoites of colpodids were counted in the first week, but the number of cysts raised constantly during this period. As in the propylene oxide experiment, the population increased significantly between days 14 and 21 ($p=0.0042$) to about 20 000 cells g^{-1} dry soil. On day 21, the number of trophozoites was lower than the number of cysts ($p=0.0056$) in the ratio of 1 to 36. Hypotrichs only

Irradiated soil (table III)

Trophozoites of colpodids were observed after 3 days, but the number of cysts increased gradually during the first week. Differences of means were never significant and the number of trophozoites was never significantly different from the number of cysts. Hypotrichs were detected at the end of the first week. Their population reached a maximum of 600 cells g^{-1} dry soil on day 28. This maximum was

Table II. – Numbers of ciliates (g^{-1} dry soil) after autoclaving and reinoculation with untreated soil. (means $\pm$ SE).

	Days					
	1	3	7	14	21	28
Colpodids						
Trophozoites	0	0	7 $\pm$ 7	18 $\pm$ 11	557 $\pm$ 309	338 $\pm$ 124
Cysts	69 $\pm$ 26	5,960 $\pm$ 3,063	20,279 $\pm$ 7,962	994 $\pm$ 516	20,242 $\pm$ 3,624	23,280 $\pm$ 12,236
Hypotrichs						
Trophozoites	0	0	0	0	0	0
Cysts	0	0	0	0	0	64 $\pm$ 46
<i>Drepanomonas</i> sp.						
Trophozoites	0	808 $\pm$ 143	738 $\pm$ 96	241 $\pm$ 125	2,549 $\pm$ 651	684 $\pm$ 112
Cysts	0	0	0	0	0	0

Table III. – Numbers of ciliates (g^{-1} dry soil) after γ -irradiation and reinoculation with untreated soil. (means $\pm$ SE).

	Days					
	1	3	7	14	21	28
Colpodids						
Trophozoites	0	43 $\pm$ 43	60 $\pm$ 33	0	110 $\pm$ 31	10 $\pm$ 10
Cysts	34 $\pm$ 15	425 $\pm$ 161	1,110 $\pm$ 397	209 $\pm$ 80	11,850 $\pm$ 6,190	252 $\pm$ 116
Hypotrichs						
Trophozoites	0	0	50 $\pm$ 10	131 $\pm$ 29	84 $\pm$ 5	157 $\pm$ 20
Cysts	0	0	68 $\pm$ 17	183 $\pm$ 41	198 $\pm$ 55	479 $\pm$ 27
<i>Drepanomonas</i> sp.						
Trophozoites	0	94 $\pm$ 47	25 $\pm$ 25	90 $\pm$ 36	18 $\pm$ 6	69 $\pm$ 44
Cysts	0	0	0	0	0	0

significantly different from the population on day 21 ($p=0.0087$). On day 28, the number of trophozoites was significantly lower than the number of cysts ($p=0.0007$) in the ratio of 1 to 3. *Drepanomonas* sp. was observed from day 3 but no significant change in the size of the trophozoite population occurred subsequently. As in the previous treatment, no cysts were counted.

DISCUSSION

When the abundances between treatments are compared, no significant difference for colpodids was detected. Only one significant difference occurred for hypotrichs on day 28 and this was larger for irradiated soil than for fumigated ($p=0.013$) or autoclaved treatment ($p=0.0009$). The numbers of *Drepanomonas* sp. (Kinetofragminophora) were always significantly larger in autoclaved soil, excepted for day 14.

Lüftenegger *et al.* (1985) suggested that the ratio of Colpodea (colpodids) to Polyhymenophora (hypotrichs) should decrease in temperate habitat. In the present experiment, this ratio was lower than 1 on days 14 and 28 in irradiated soil while it was constantly higher in fumigated. Therefore, the irradiated soil could be assumed to be a "stable" habitat, at least more than the other treatments. Furthermore, the community was most diversified in the irradiated soil where 10 species were found compared with 6 in the other two treatments (Palka, 1991). Thus, the more environmental conditions are "stable" the more species number is higher. This is in accordance with the theory of Thienemann (Dajoz, 1970).

The community development was marked by different stages. First bacteriophagous ciliates appeared (Foissner, 1987) "indifferent" and "semi-sensitive" to

the biotope conditions (Palka, 1991). Colpodids were numerically dominant in each treatment, especially as cysts. They are well adapted to the changes of habitat (Lüftenegger *et al.*, 1985). However, the increase of cyst numbers in the autoclaved treatment during the first week is difficult to interpret. The growth of *Drepanomonas* sp. was relatively rapid (table II) but the absence of encysted forms is very surprising because encystment is often assumed to be an adaptation to the life in soils (Foissner, 1987). This result must be confirmed in studying the behaviour of this species in different culture conditions. However that may be, this example of "semi-sensitive" form shows that the degree of sensitivity to the changes of habitat is probably not connected to the quantity of cysts formed.

The second stage coincided with the growth of omnivorous consumers (hypotrichs) "sensitive" to the biotope conditions (Palka, 1991) and predators of other ciliates, green algae or rotifers (Foissner, 1987). Hypotrichs are characteristic of undisturbed soils and they have been described as more K-selected than colpodids (Lüftenegger *et al.*, 1985). However, *Gonostomum affine* is bacteriophagous and "indifferent" to the changes in the habitat caused by sterilization (Palka, 1991). But it usually appears later in cultures than colpodids, again indicating that it is a little more K-selected than colpodids.

In conclusion, this laboratory experiment has described three different responses of the ciliate community to the changes in soil caused by propylene oxide fumigation, autoclaving or γ -irradiation. Responses of the ciliate community could also be studied in forest reserves, according to the changes in soil litter induced by the different stages of tree regeneration.

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